(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 407 800 A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 90112082.4

(22) Date of filing: 26.06.90

(51) Int. Cl.5: G01N 33/52, //C12Q1/00, C12Q1/54

(30) Priority: 08.07.89 DE 3922496

(43) Date of publication of application: 16.01.91 Bulletin 91/03

(84) Designated Contracting States: DE FR GB IT

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(54) Analysis method for determining substances from biological fluids.

(57) A test strip for analyzing subetances from biological fluids is disclosed in which the matrix comprises a porous membrane having an asymmetric pore structure which is designed for application of the biological fluid to the large pore side of the membrane and in which a determination of the biological fluid is made on the opposite side of the membrane containing a small pore size. The membrane contains anionic surfactant in an amount of 1 to about 4% by weight based on the polymer casting solution used to form the porous membrane.

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ANALYSIS METHOD FOR DETERMINING SUBSTANCES FROM BIOLOGICAL FLUIDS

Field Of The Invention

The present invention relates to test strips or test devices for analyzing substances from biological fluids in which the matrix comprises a porous membrane having asymmetric pore structure and designed for the application of the biological fluid to the large pore side of the membrane and making a determination on the opposite fine pore side of the membrane. The membrane contains anionic surfactant equivalent to surfactant addition of about 1 to about 4 percent by weight based on polymer casting solution.

Background Of The Invention

Test strips which contain reagents in a matrix of paper or plastic material and in which the sample is applied directly to this matrix have become extremely important for quick and simple analysis of individual samples. Measurement results which are the same as or better than wet chemical methods can be obtained.

However, the test strips commercially available to date for determining blood constituents frequently possess certain disadvantages. The erythrocytes contained in blood interfere with most methods. The user must therefore typically remove serum or plasma from whole blood by centrifuging before making an analysis. This, however, presents problems, particularly in the case of small sample amounts. With more recent testing agents the reagent layer itself (European Patent 0 084 710, DOS German Published Specification 34 07 395) or a covering membrane is semipermeable and retains the erythrocytes. Accordingly, the test systems can be directly loaded with whole blood. However, in these test systems hemoglobin has to be removed by wiping or washing off before reflection-photometry or visual analysis. This method cannot be used for analysis of large molecules, e.g., certain enzymes, because these are also retained by the semipermeable layer. In addition, the wiping-off process constitutes a potential source of dangerous infections because blood samples may occasionally be contaminated with hepatitis viruses or other pathogens.

In view of the requirement for high reproducibility of the diagnosis, test strips systems which have a very simple structure and are uncomplicated to produce, with a few production steps, are preferred.

It is occasionally also desirable to have a delay phase between application of the sample and start of the reaction so that the temperature of the sample liquid can be stabilized before any subsequent reactions are carried out.

Finally, it is desirable to be able to use the same matrix for analysis of blood and urine. In fact, as described in detail in EP-A 84 710 and DE 38 90 523.8, the previously known microporous blood matrices cannot be used directly, without further aids, for a dip-and-read test in urinalysis.

There is as yet no test strip system which fulfills all these requirements simultaneously.

There has been no lack of attempts aimed at developing test strips for blood analyses in which separation of the erythrocytes or hemoglobin is simultaneously accomplished in the system.

DE-AS (German Published Specification) 22 22 951 or U.S. Patent No. 4,144,306 describes multilayer test devices for blood in which one or more of these layers act as filters which retain the corpuscular constituents of blood. Membranes having pores of 1-3 µm are used for separation of the erythrocytes. These pores easily block and impede passage of plasma with the result that the plasma enters the reaction layer slowly and nonuniformly.

Progress was achieved by using special glass fiber filters as described in DE-OS (German Published Specification) 30 29 579.5. On application of blood the particulate constituents of blood are retained while the plasma is transported to the reagent layer in which the detection reaction takes place. However, the dead volume of this erythrocyte retention zone is relatively high. The ratio of separated plasma to dosed blood therefore becomes even worse. Thus, it can occasionally be the case that the separation system cannot cope with the quantity of erythrocytes to be separated off, so that blood pigment reaches the reaction zone and causes interference there.

Special retention systems are described in European Patent 0 133 895 for further improvement of this glass fiber system. These are, for example, papers impregnated with polar compounds, e.g., special dyes. These substrates effect strong coagulation of blood, so that the corpuscular constituents are separated more effectively from the serum.

As described in European Patent 0 133 895, there is no universal retention substrate which is equally

PAGE 6/10 * RCVD AT 11/29/2005 3:53:16 PM [Eastern Standard Time] * SVR:USPTO-EFXRF-6/25 * DNIS:2738300 * CSID:317 637 7561 * DURATION (mm-ss):09-18

suitable for each test. For example, some substances cause hemolysis or undergo undesirable secondary reactions with enzymes. It is therefore necessary to determine the most favorable retention substrate for each test. In addition, some of the analytes to be determined can be entrapped when the blood coagulates. The structure of the systems described in European Patent 0 133 895 is fairly complicated. Thus, the system for detection of glucose consists, for example, of seven individual elements. The reaction is triggered by the polymer matrix, which is provided with the detection reagents, being pressed onto the transport wadding filled with plasma. Since an oxygen deficiency problem would arise in oxidation reactions in the compressed state specific reagent matrices have been developed for this system and are described in European Patent 0 113 898. These are film layers containing reagents on a multifilament fabric carrier layer or wadding. The reagent films are prepared, as described in this application, from aqueous polymer dispersions in the presence of fillers. Since a certain amount of oxygen is stored in the multifilament fabric layer an oxygen-consuming reaction can take place in the above system even in the compressed state.

A disadvantage of this system is that the oxygen content is limited and, as described in European Patent 0 113 898, is insufficient for the oxygen requirements essential for the reaction. The reaction layers on multifilament fabric layers described in this specification are used in combination with the above-mentioned erythrocyte retention substrates. Erythrocyte separation inherent to the system is evidently impossible with these reagent layers supported by wadding or fabric. As mentioned there, the reagent layers are prepared from polymer dispersions in the presence of fillers by the methods described in DE-AS (German Published Specification) 15 98 153, DE-OS (German Published Specification) 29 10 134 and DE-OS (German Published Specification) 31 18 381. The resulting pore structures have very small pore diameters, low porosities and no pore through-channels.

European Patent Applications 0 110 173 and 0 256 806 of Lifescan describe detection elements which have a considerably simpler and less complicated structure and which can be used directly for whole blood analysis. As described in the last-mentioned specification, the reagent matrix is hydrophilic polyamide membrane (nylon) impregnated with detection reagents.

A noteworthy feature of this system, which is already commercially available, is that the red blood pigments are not separated. On application of blood the entire reagent matrix becomes red. The glucose color reaction is evaluated with a device whose wavelength does not interfere with the red blood pigment but which detects the chromogenic reaction. However, these systems cannot be used for visual evaluations. As shown by analyses of this reagent matrix under the electron microscope, there are highly porous membrane layers located on either side of a polymer wadding. The pore structure is symmetrical and has diameters of 2 to 12 µm (microns). There can be no separation of erythrocytes. By contrast, it is described in the same application (EP-A 0 256 806) that membranes having mean pore diameters of 0.1 to 2.0 µm are preferably used for blood analyses. The above-mentioned difficulties apparently also occur with this system, i.e., that porous systems having pore diameters of less than 3 µm separate the erythrocytes but are blocked by high molecular compounds and, moreover, do not allow high molecular analytes to pass, whereas precisely the opposite is the case with membranes having much larger pores.

Summary Of The Invention

It is an objection of the present invention, then, to develop a test strip system and an associated method of analysis whose construction and manipulation are as uncomplicated as the last-described system but which at the same time allows separation of red blood pigments (cells) without any additional aids (coagulants). In addition, it should be possible to precede the actual detection reaction, where necessary, by specific preliminary reactions, for example, to remove interfering components, it being possible for the detection and preliminary reactions to be separated in time, where necessary, by timing delays. Finally, it should furthermore be possible to use the detection elements for a dip-and-read test in urinalysis.

It has now been found, surprisingly, that membranes having an asymmetric pore structure into which the corresponding detection reagents are incorporated can be employed in an outstanding manner to achieve the aforementioned objects. The expression "asymmetric pore structure" is familiar to membrane experts and is described in detail in a large number of publications.

DOS 34 07 369 and U.S. Letters Patent No. 4,774,192 disclose a form of asymmetric membrane pore structure. However, in contrast to the claimed invention, sample is applied to the small pore side of the membrane. This is a sample receptive surface which the '192 patent described as "essentially impermeable to cells and particulate matter". Accordingly, in the case of blood and other biological samples the pores of the membrane become clogged very quickly rendering the membrane ineffective as a reagent matrix material for use with biological materials such as whole blood. Such detection systems are unsuitable for

the application purpose according to the invention, and are suitable only for wipe-off systems in which the blood has to be wiped off.

As described herein deficiencies of the prior art with respect to color gradation and color stability were overcome by the addition of certain anionic surfactants in particular amounts. These particular surfactants and the critical amounts of the surfactants are not disclosed by the '182 patent.

Two other publications which can be mentioned which refer to asymmetric pore structure and membranes are the following: H. Strathmann, "Trennung von molekularen Mischungen mit Hilfe synthetischer Membranen", (Separation of molecular mixture by means of synthetic membranes) Steinkopf-Verlag, Darmstadt 1979 and D. R. Lloyd, "Materials Science of Synthetic Membranes", ACS Symposium 269, Washington, D.C. 1985. The most important method of preparing such membranes, namely the precipitation coagulation method (also called phase inversion), is also mentioned. The integral asymmetric pore structure with a dense polymer structure, also called a polymer skin or active separating layer, at the membrane surface and larger pores and higher porosity in the underlying highly porous support layer are typical of such membranes.

Whereas the thickness of the active separating layer is about 0.2-2.9 µm, the underlying highly porous support layer is as a rule about 20 to 100 µm thick, depending on the wet application during production of the membrane. The structure of the highly porous support layer can possess a foam-like or finger-like structure or even a mixed structure of these two, depending on the polymer and the preparation method.

Subsequent modification or preparation with a further, e.g., even denser, active separating layer on the original active separating layer by the so-called composite method (J.E. Cadotte, ACS Symposium Ser. 153 [1981], 305-326) is also possible, but less preferred, for the preparation of the detection elements according to the invention.

The membranes used for preparation of the detection elements according to the invention can either be carrier-free or can adhere with the lower side of the membrane on a permeable carrier material customary for technical membranes, for example, polymer wadding or multifilament fabric. If suitable detection reagents are incorporated in such membrane systems and exposed to whole blood on the lower side of the membrane, the red blood pigments are separated in the membrane while a color reaction, without erythrocyte interference, can be observed on the opposite membrane side.

The pore size on the lower side of the membrane (application side) should be at least 3 µm, preferably 5 to 10 µm. the pores on the opposite upper side of the membrane should be not larger than 1 µm. Pores having a diameter of 0.1 to 0.5 µm are preferred.

The special pore structure of these membrane matrices is evidently of decisive significance for the detection elements according to the invention. As shown by SEM photographs the mechanisms of these systems can be interpreted as follows. Whole blood flows unimpeded through the large pores (>5 µm) on the application side of the membrane while plasma separation takes place through the increasing pore narrowings in the active separating layer.

By contrast, low molecular compounds including the low molecular products of the detection reaction, such as hydrogen peroxide, can pass through the microporous active separating layer.

If, then, a chromogenic detection system is introduced into the active separating layer or onto its surface, a detection reaction without interference from erythrocytes can be observed at the membrane surface after application of blood onto the reverse side of the membrane.

It is also possible to use other detection methods, for example electrochemical detection methods, instead of a chromogenic detection system because membranes, as described in DE-OS (German Published Specification) 36 15 831, can be easily metallized and therefore made electrically conductive.

Brief Description Of The Drawings

Other and further objects, advantages and features of the invention will be apparent to those skilled in the art from the following detailed description thereof, taken in conjunction with the accompanying drawings in which:

Fig. 1 is a diagrammatic cross-sectional view of a portion of a reagent matrix membrane usable in accordance with the present invention;

Fig. 2(a) and (b) are a diagrammatic perspective view of a test strip in accordance with the present invention and a cross-sectional side view of the same test strip, respectively;

Fig. 3 is a side view of a test strip in accordance with another embodiment of the present invention;

Fig. 4(a) and (b) are diagrammatic side views of two other embodiments of the present invention;

Fig. 5 is a series of four drawings illustrating in sequence the preparation of the test strip embodiment

illustrated in Fig. 3 [Figs. 5(a) through (o)] and in Fig. 4(a) [Fig.5d];
Fig. 6 is a side view of yet another embodiment of the present invention;
Fig. 7 is a diagrammatic perspective view of a test strip in accordance with the present invention in which a color chart is positioned on the test strip in the vicinity of the location where visual readings are made;
Fig. 8 is a series of five drawings which illustrates the preparation of yet another test strip in accordance with the present invention by sequential illustrations (a) through (d), which lead to the preparation of the embodiment shown in side view Fig. 8e; and
Figs. 9 to 11 are diagrammatic side views of three additional embodiments of the present invention, all of which contain an opening in the substrate beneath the reagent matrix for visualization of the color change which occurs in the reagent matrix following application of sample to the test strip.
In the figures the last digit of an item number is identical for similar parts in different figures.

Description Of The Preferred Embodiments

An important advantage of the detection elements described here is the relative ease with which they can be prepared. For example, the membrane systems shown in Figure 1 can be prepared according to the present state-of-the-art in a single operation (coating a carrier material with a polymer casting solution followed by coagulation and drying). In the diagrammatic representation of the membrane in Figure 1(A) is the active separating layer which usually has a thickness of 0.2 µm to 2.9 µm; (B) is the integrally asymmetric polymer membrane which is normally 50 µm thick; and (C) is a layer of material which can be a fabric or a wadding, the thickness of which is about 10 to 250 µm.

The reagents necessary for the detection reaction can be incorporated, as described in detail in DOS (German Published Specification) 34 07 358, in the membrane either directly via the polymer casting solution or by subsequent impregnation. The latter is preferred in the case of water soluble reagents. A combination of these two methods is used in many cases, organic soluble reagents being incorporated via the polymer casting solution and water soluble substances, such as enzymes, being incorporated by impregnation. Impregnation is preferably carried out with the extruder or cascade method described in EP-A 246 505.

A further advantage of the detection elements described here is the wide selection of possible polymers. Virtually all soluble polymers are suitable for preparation of the membrane by precipitation coagulation, also called phase inversion. Thus, there is a selection of hydrophilic, hydrophobic, neutral, cationic and anionic polymers which allows adjustment to the particular detection system.

Examples of suitable polymers include: polyvinylidene fluoride, polysulphone, polyhydantoine, styrene/maleic anhydride copolymer, polyamides, cellulose acetates, polyethers, polycarbonates, polyurethanes, where appropriate in combination with ionic polyurethane dispersions, polyacrylonitrile and copolymers thereof. Acrylonitrile copolymers with ionic, in particular cationic, groups are preferred.

Acrylonitrile copolymers with anionic functions, betaine structures or mixtures of cationically and anionically modified copolymers can also be employed. Example 1 shows the chemical structure of a preferred cationic acrylonitrile polymer.

The acrylonitrile copolymers contain at least 50 percent by weight, preferably at least 80 percent by weight, of acrylonitrile.

Suitable copolymerized comonomers are both neutral comonomers and, as mentioned, comonomers having anionic, cationic or betaine functions.

Examples of neutral monomers which can be present in a polymer, alone or in combination with one another, in amounts of 0.5 to 50 percent by weight, are, along others, methacrylonitrile; (meth-)acrylic esters, e.g., methyl, ethyl, butyl, hexyl, 2-ethylhexyl, cyclohexy, hydroxyethyl, hydroxypropyl, ethoxyethyl, butoxyethyl, methoxyethyl, phenyl, phenylethyl, phenylpropyl, furfuryl, tetrahydrofurfuryl, polyalkylene ether (meth)acrylate; vinyl esters such as vinyl formate, vinyl acetate, vinyl propionlate, vinyl butyrate, vinyl benzoate; (meth-)acrylamide and N-mono- or N,N-dialkyl- or hydroxy- and alkoxyalkyl derivatives thereof, e.g., dimethyl(meth)acrylamide, N-hydroxymethyl-, N-methoxymethyl(meth)acrylamide; maleic acid amide; itaconic acid amide; unsaturated ketones such as methyl vinyl ketone, phenyl vinyl ketone, methyl isopropenyl ketone; diacetone acrylamide; vinyl ethers such as vinyl methyl ether, vinyl ethyl ether; N-vinylcarboxyamides such as N-vinylformamide, N-vinylacetamide, N-vinyl-N-methylformamide, N-vinyl-N-methylacetamide; N-vinyllactams such as N-vinyl-pyrrolidone, N-vinylcaprolactam; styrene; α-methylstyrene; and diisobutene.

Suitable anionic monomers which can be polymerized with acrylonitrile alone or in combination with

neutral monomers are unsaturated carboxylic acids, e.g., (meth)acrylic acid, itaconic acid, maleic acid, fumaric acid and the corresponding salts. The maximum content of free carboxylic acid in the polymer can